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Effects of Interactions between Host Plants and Selective Insecticides on Larvae of *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) in the Laboratory

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Abstract: The residual toxicity of two selective insecticides, teflubenzuron (acylurea) and *Bacillus thuringiensis* Berliner ssp. *aizawai* (microbial), to laboratory and field strains of *Plutella xylostella* L. was shown in the laboratory to be significantly affected by leaf nutritional status, other host-plant resistance factors, cultivation method and plant age. With plants offering some degree of host-plant resistance, the toxicity of the insecticides was either increased or decreased compared with highly susceptible plants, depending on the specific nature of the plant–herbivore interaction. Differences in residual toxicity of the insecticides varied up to nine-fold on different host plants (=host-plant- + insecticide-induced mortality) despite less than four-fold differences in *P. xylostella* mortality in controls (=host-plant-induced mortality alone). Host-plant nutritional status also had a substantial effect on the damage potential of larvae. Different response times by *P. xylostella* to the two insecticides tested on host plants of varying nutritional status were related to the contrasting modes of action of the respective active ingredients. The present studies suggest that insecticides applied to *Brassica oleracea* L. var. *capitata* with partial plant resistance may contribute to improved control of *P. xylostella*. A conceptual model is used to describe likely mortality responses by macrophagous larvae to insecticides applied to plants of varying resistance status. The implications of the findings in relation to the integrated management of *P. xylostella* are considered.

Key words: *Bacillus thuringiensis*, acylurea, plant resistance, diamondback moth.

1 INTRODUCTION

The potential of synergistic interactions between host-plant resistance and insecticides (at reduced doses) in commercial agriculture has yet to be fully exploited. Indeed, studies on interactions between plant resistance and insecticides have often yielded conflicting results,[1] suggesting that there are no consistent or general rules which apply to plant resistance–insecticide interactions. Increased levels of plant resistance have been shown to increase[2–7] or decrease[8–10] the susceptibility of a given insect population to an insecticide under specific conditions. Other workers have found that differing levels of host-plant resistance have had no effect on insecticide susceptibility.[11] When the toxicity of abamectin to *Plutella xylostella* L. was evaluated on *Brassica oleracea* L. (cabbage) cultivars of varying host status, the insecticide was generally more toxic on the more resistant varieties when applied topically to larvae, while the situation was reversed when residual deposits were ingested in the leaf-dip assays.[12]

Possible reasons for variable results with particular toxicants in these types of study are numerous and may include differences in insect feeding rate, behaviour and enzyme enhancement as well as differential bioavailability and host nutritional status on different host plants/cultivars (Section 4). Different factors or combinations of factors are likely to be involved in different herbivore/host plant/insecticide interactions.

The present studies employ a laboratory leaf-disc bioassay technique with the aim of assessing the possible

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impact of different host plants/cultivars on the residual toxicity of two selective insecticides, the acylurea insect growth regulator teflubenzuron ('Nomolt'®) and the microbial biopesticide *Bacillus thuringiensis* (*Bt*) Berliner ssp. *aizawai* ('Florbac'®) to laboratory and field strains of *P. xylostella*. The relationship between dose of the same two products and leaf surface area damage by larvae from a field strain of *P. xylostella* was also examined.

2 MATERIALS AND METHODS

2.1 Plants

Five cultivars of *B. oleracea* var. *capitata*. (cvs Wheelers Imperial, Minicole F_1, Winnigstadt, Greyhound and Red Drumhead; Suttons Seeds Ltd, Torquay, UK) and a single *B. pekinensis* (Lour.) Rupr. cultivar (cv. Tip Top; Chiltern Seeds, Ulverston, UK) were used in experiments. Plants were sown in seed trays in the glasshouse and transferred to pots (13 cm dia.) containing a standard potting mix (Levingtons Multipurpose Compost®, Fisons, UK) at the five- to six-leaf stage. Seedlings were either maintained in pots in the glasshouse (*glasshouse-grown plants:* see below) or placed outdoors (*outdoor-grown potted plants:* see below). Potted plants required for more than 90 days were transferred to large pots (26 cm dia.) containing a soil-based compost (John Innes No. 3®, J. Arthur Bowers, Lincoln, UK). *Glasshouse-grown plants:* potted plants were maintained on moist capillary matting and were exposed to supplementary lighting (400-W poot lamps suspended *c.*2 m above compost surface) for 12 h day^{-1}. Thermostatically- controlled heating prevented glasshouse temperatures falling beneath 15°C in winter, but maximum temperatures in summer sometimes exceeded 30°C. No additional fertilizer was added with any cultivation method. The glasshouse was occasionally fumigated with nicotine (DowElanco 40% w/w Nicotine Shreds®) during the summer months to disinfest plants of aphids, and plants were not used in experiments within 96 h of such treatments. *Outdoor-grown potted plants:* potted plants were placed in a grid (with *c.*45 cm spacing between plants) in an unshaded location at Silwood Park. They were watered daily and no insecticides were applied to the plants, although molluscicidal pellets (ICI 'Slug Xtra'® containing 40 g kg^{-1} metaldehyde) were placed on the ground around pots to control slugs and snails.

2.2 Insect culture

Two strains of *P. xylostella* were used: a sub-population (ROTH) of a long-term laboratory strain from the Institute of Arable Crop Research, Rothamsted (Harpenden, UK) and a field strain (SERD 2) collected in Serdang, Malaysia in late 1993 by Dr Dzolkhifli Omar (Universiti Pertanian Malaysia) and subsequently maintained in the laboratory in Malaysia and then Silwood Park for a total of 18 generations prior to use. Cultures and experiments were maintained in constant environment rooms at 20(±2)°C and 65(±3)% RH under a 16 : 8 h light : dark cycle. Cultures were generally reared on six- to eight-week-old glasshouse-grown Chinese cabbage (cv. Tip Top) but were reared for at least one generation on specific plant groups prior to testing on the same respective plant groups. Larval instars were identified by the width of the head capsule.[13]

2.3 Chemicals

The two insecticides tested were the acylurea (chitin synthesis inhibitor) teflubenzuron ('Nomolt'®; 50 g $litre^{-1}$ suspension concentrate (SC; Shell Research Ltd, Sittingbourne, UK) and the microbial biopesticide *Bt* ssp *aizawai* ('Florbac'®; 8500 iu mg^{-1} wettable powder (WP); Novo Nordisk, Bagsvaerd, Denmark). Test dispersions were freshly prepared in distilled water with addition of the surfactant 'Triton' X-100 to improve coverage of the solution ('wetting') on the waxy surfaces of the *Brassica* leaves tested (refer to specific experiments for concentrations of 'Triton' X-100 used).

2.4 Leaf-dip bioassay technique

A sharpened metal hole-punch was used to cut leaf discs (4·8 cm dia.) from middle or inner leaves of selected plants. Each leaf disc was immersed for 10 s in a beaker containing the appropriate test solution, then suspended for a further 10 s over the beaker to allow run-off of any surplus solution. Discs were then transferred to corrugated sheets of aluminium foil, with the adaxial leaf surfaces upwards, for drying at room temperature. Once dry, the leaf discs were transferred to individual plastic Petri dishes (5 cm dia.) containing filter paper (Whatman No. 1; 4·5 cm dia.) moistened with *c.* 20 μl distilled water. Five two-day-old second-instar larvae of *P. xylostella* were transferred to each Petri dish. Each treatment was replicated six times. After five days, the remains of the host-plant leaf disc were removed from each Petri dish and fresh leaf discs from the same host plant/cultivar/age group were added *ad lib.*

2.5 Residual toxicity of insecticides to a laboratory strain of *Plutella xylostella* when applied to leaf discs from six plant groups

Leaf discs cut from six host plant/cultivar/age groups were tested in leaf-dip bioassays (Section 2.4) with three concentrations of teflubenzuron (0·016, 0·05 and 0·15 μg AI ml^{-1}) and *Bt* ssp. *aizawai* (0·11, 0·33 and 1 iu mg^{-1}), against a laboratory strain (ROTH) of *P. cylostella*. *B. pekinensis* was tested (treatments and dis-

tilled water controls) with two concentrations of surfactant (50 and 100 μl 'Triton' X-100 ml^{-1}), whereas only the higher rate of surfactant was used for the remaining plants (all *B. oleracea* var. *capitata*). The higher rate of 'Triton' X-100 was found to be necessary on the *B. oleracea* leaf discs in order to ensure that the leaf surfaces, which were noticeably more waxy than those of the *B. pekinensis* cultivar tested, were wetted evenly following dipping. Each of the six selected plant groups was represented by a different combination of host plant species/cultivar/cultivation method/age. Mortality was assessed after 5, 9 and 12 days.

2.6 Residual toxicity of insecticides to laboratory and field strains of *Plutella xylostella* on standard age classes of host plants/cultivars

The protocol was as in Section 2.5, except that in experiments with the laboratory strain (ROTH), three glasshouse-grown host plants/cultivars (*B. pekinensis* cv. Tip Top, and *B. oleracea* var. *capitata* cvs Wheelers Imperial and Red Drumhead) of standardised age (107 days from sowing) were used. 'Triton' X-100 was added at twice the concentration for leaf-dips with *B. oleracea* compared with *B. pekinensis* (see above).

A similar experiment with the field strain (SERD 2: F_{18}) was set up using two age classes (84- and 147-day-old) of cv. Wheelers Imperial and Red Drumhead (both *B. oleracea*). For this latter experiment, the concentration used for teflubenzuron were 0·02, 0·67, 2·00 and 6·00 μg AI ml^{-1} and for *Bt* ssp. *aizawai* were 0·04, 0·11, 0·33 and 1·00 iu mg^{-1}. Experiments with the field strain also included visual assessments of leaf-disc damage at day 5. Percentage damage to the surface area of each leaf disc was visually assessed according to the following rating scale: 0 = <5%, 1 = 5–25%, 2 = 26–50%, 3 = 51–80% and 4 = 81–100%.

2.7 Statistical analysis

Results were analysed in one or more of the following ways:

(a) Comparison of mortalities at median doses. Data were modelled in GLIM[14] with the aim of finding consistent trends between host-plant-induced and host-plant + insecticide-induced mortality.

(b) Logit analysis. Bioassay data were corrected for control mortality according to the method of Abbott,[15] except in the experiments with the field strain, where no highly susceptible plant groups were included and control mortality consequently exceeded 10%. Estimates of LC_{50} values and their 95% fiducial limits (FL) were obtained by maximum likelihood logit regression analysis in GLIM using the logistic model and generalised linear modelling techniques.[14] Tests for parallelism between sets, where appropriate, were calculated by analysis of deviance ($P = 0·05$ rejection level). Differences between LC_{50} values of sets were determined *via* the corresponding 95% FLs.

(c) Regression analysis (using binomial errors) and model optimisation in GLIM[14] for interpreting the relationship between toxicant dose and leaf damage.

3 RESULTS

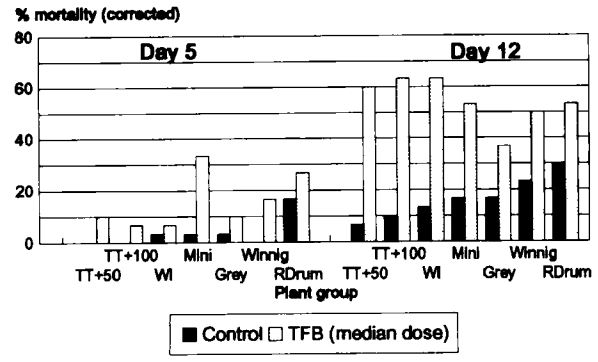
3.1 Residual toxicity of insecticides to a laboratory strain of *Plutella xylostella* when applied to leaf discs from six plant groups

Host plant/cultivar group mediated substantial effects on the residual toxicity of the two insecticides (median doses) to the laboratory strain of *P. xylostella*, contributing to up to seven-fold differences in mortality (Fig. 1). Logit analysis of the more complete data showed that, for both insecticides, the plant group with highest nutritional status[16] (i.e. lowest control mortality) to *P. xylostella* (=*B. pekinensis* cv. Tip Top) yielded the greatest larval mortality (Table 1). The higher concentration of 'Triton' X-100 significantly ($P < 0·001$) enhanced the toxicity of the *Bt* product, whereas 'Triton' X-100 had no significant ($P > 0·05$) effect on the toxicity of teflubenzuron. When the percentage mortality (arcsine transformed) caused by the median dose treatment for teflubenzuron and *Bt* ssp. *aizawai* with the six plant groups was regressed against control mortality for the same plant groups (explanatory variable), the slopes were not significantly ($P > 0·05$) different. The r^2 (coefficient of determination) values were 0·11 and 0·02 for teflubenzuron and *Bt* ssp. *aizawai* respectively. The non-linear relationships between control and treatment responses are shown in a scatterplot of the transformed data (Fig. 2); although a trend in the teflubenzuron data is apparent, no discernible trends are found with the *Bt* ssp. *aizawai* data.

3.2 Residual toxicity of insecticides to a laboratory strain of *Plutella xylostella* on a standard age class of host plants/cultivars

When the residual toxicities to the ROTH strain of *P. xylostella* on three plant groups of equivalent age were compared by means of LC_{50} values, both products were significantly ($P < 0·05$) more toxic to *P. xylostella* (ROTH) on cv. Wheelers Imperial and cv. Tip Top than on cv. Red Drumhead (Table 2). There were no significant ($P > 0·05$) differences between LC_{50} values derived from either cv. Tip Top or cv. Wheelers Imperial with either insecticide.

A. Teflubenzuron



B. *Bt* ssp. *aizawai*

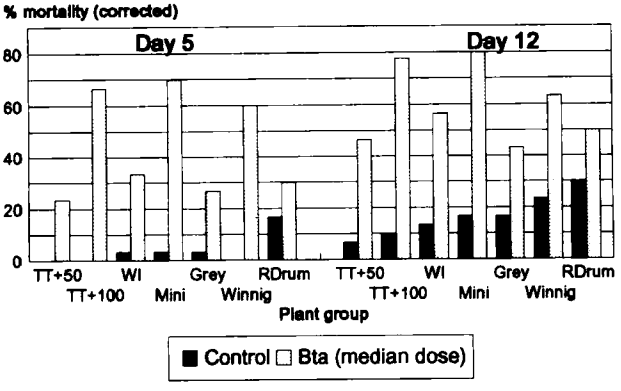


Fig. 1. Residual toxicity of median doses[a] of teflubenzuron and *Bt* ssp. *aizawai* to second-instar *Plutella xylostella* (laboratory strain) reared and maintained on six plant groups[b]
[a] Teflubenzuron = 0·05 μg AI ml^{-1}: *Bt* ssp. *aizawai* = 0·11 iu mg^{-1}: in leaf-dip bioassays.
[b] Plant groups (one with two surfactant concentrations): TT + 50 (+100) = cv. TipTop + 50 μl Triton X-100 ml^{-1} (+100 μl Triton X-100 ml^{-1}): WI = cv. Wheelers Imperial: Mini = cv. Minicole F_1: Grey = cv. Greyhound: Winnig = cv. Winnigstadt: RDrum = cv. Red Drumhead.

3.3 Residual toxicity of insecticides to a field strain of *Plutella xylostella* on two age classes of two cabbage cultivars and the effect on leaf damage

Relative toxicities of the two toxicants to the SERD 2 field strain of *P. xylostella* varied by up to nearly nine-fold between plant groups in the case of teflubenzuron and more than five-fold for the same plant groups with *Bt* ssp. *aizawai* (Table 3). With both insecticides, an intermediate plant group (based on relative control mortalities) contributed to the lowest residual toxicity, with substantially greater toxicities resulting on both the most susceptible (cv. Wheelers Imperial, 84 days old) and least susceptible (cv. Red Drumhead, 147 days old) (Table 3).

The relationship between larval damage to the surface of leaf discs from the four plant groups and the concentration of teflubenzuron and *Bt* ssp. *aizawai* is shown in Fig. 3 and a summary of the regression data is given in Table. 4. Significant ($P < 0·05$) regression slopes were found for all plant groups with teflubenzuron but, despite reasonable r^2 values, the slopes for the *Bt* ssp. *aizawai* data were not significantly different from zero (ie. $P > 0·05$) (Table 4). The data show that it is highly (95%) probable that there is a direct correlation (described by the model given in Fig. 4) between the rate of leaf damage and concentration of teflubenzuron and, in the case of *Bt* ssp. *aizawai*, that this relationship is equally likely to be non-significant.

4 DISCUSSION

The majority of work concerning interactions between plant resistance and insecticidal control of insect pests appears to have been confined to microphagous pests, particularly aphids.[2,3,5] A recent study by van den Berg *et al.*[7] represents one of few studies relating to macrophagous pests. These authors reported that insecticide efficacy against the stem borers *Chilo partellus* Swinh. and *Busseola fusca* Full. on susceptible and resistant lines of sorghum was significantly greater on resistant lines and this was attributed to larval stress caused by antibiosis and antixenosis in the resistant plants.

The present work, which includes interactions between plant resistance (in *B. oleracea* var. *capitata*) and certain insecticides as well as between different host plants (*B. oleracea* var. *capitata* and *B. pekinensis*), demonstrates the variable nature of such interactions. The laboratory studies, based on leaf-dip bioassays, represent a rather artificial scenario when compared with outdoor-grown whole plants subjected to field application of insecticides and natural infestation by pests, but did allow standardisation of factors which might otherwise have masked important biological trends in the interactions. Key factors of importance in the interaction between insecticides, host plants and macrophagous insect pests are likely to include:

- Bioavailability of the insecticide(s) on different plant surfaces.
- Inherent susceptibility (lack of fitness) of the herbivore.
- Rate of foliar consumption.
- Ratio of insecticide treated: untreated leaf material consumed.
- Insecticide dose in relation to insect body weight.
- Mode of action of insecticide.
- Host plant resistance mechanism(s).

The apparent differences in the time–mortality response to *Bt* ssp. *aizawai* and teflubenzuron observed in the first experiment (Fig. 1 and Table 1) are likely to be attributable to the differences in mode of action between the two products. Teflubenzuron, a chitin syn-

TABLE 1

Logit Analysis[a] of Mortality Data for a Laboratory Strain (ROTH) of *Plutella xylostella* Reared and Maintained on Six Plant Groups[b] and Exposed to Teflubenzuron and *Bt* ssp. *aizawai* in Leaf-Dip Bioassays

Insecticide *Host plant* *Cultivar*	*Plant age (days)*	*Cultivation methods*[c]	LC_{50}[d,e]	*95% FL*	*Slope*[e] (±*SE*)	*RT*[f]
Teflubenzuron						
B. pekinensis						
TipTop + 50 µl TX ml^{-1}	50	GH	0·05a	0·03–1·00	2·13 (±0·59)a	3·4
TipTop + 100 µl TX ml^{-1}	50	GH	0·05a	0·03–0·11	1·96 (±0·58)a	3·4
B. oleracea var. *capitata*[g]						
Wheelers Imperial	85	GH	0·16b	0·07–13·27	1·40 (±0·57)a	1·2
Minicole F_1	85	GH	0·08ab	0·04–0·35	1·59 (±0·57)a	2·2
Red Drumhead	85	GH	0·08ab	0·04–0·52	1·43 (±0·56)a	2·2
Winnigstadt	236	F	0·10b	0·06–0·24	2·32 (±0·60)a	1·8
Greyhound	236	F	0·18b	0·09–2·64	1·71 (±0·60)a	1
Bt ssp *aizawai*						
B. pekinensis						
TipTop + 50 µl TX ml^{-1}	50	GH	0·14b	0·09–0·19	3·39 (±0·85)a	1·6
TipTop + 100 µl TX ml^{-1}	50	GH	0·03a	0·00–0·10	1·87 (±0·87)a	7·3
B. oleracea var. *capitata*[g]						
Wheelers Imperial	85	GH	0·11b	0·07–0·14	3·97 (±1·13)a	2·0
Minicole F_1 [h]	85	GH	(<0·1)	—	—	—
Red Drumhead	85	GH	0·22c	0·15–0·32	2·93 (±0·77)a	1
Winnigstadt	236	F	0·11ab	0·03–0·17	2·72 (±0·88)a	2·0
Greyhound	236	F	0·19c	0·13–0·27	3·33 (±0·79)a	1·2

[a] Control mortalities for all analyses based on those for TipTop + 100 µl 'Triton' X-100 (TX) ml^{-1} end-point day 12.

[b] As for Fig. 1 and Table 1.

[c] GH = glasshouse-grown: F = field-grown in pots.

[d] Units: µg AI ml^{-1} for teflubenzuron; iu mg^{-1} for *Bt* ssp. *aizawai*.

[e] For each toxicant, values followed by a common letter are not significantly ($P > 0·05$) different (five insects/replicate: six replicates/treatment: $n = 30$).

[f] Relative toxicity factor for each insecticide: toxicity (measured by LC_{50}) of specific plant/insecticide combination in relation to least toxic plant/insecticide combination (=cv. Greyhound for TFB and cv. Red Drumhead for *Bta*).

[g] Test solutions for all *B. oleracea* cultivars contained 100 µl TX = 100 ml^{-1}.

[h] Excessive responses at doses tested prevented logit analysis.

thesis inhibitor, significantly extends the larval duration, and mortality does not occur until the insect undergoes ecdysis following accumulation of a lethal dose.[13] This teflubenzuron-induced developmental delay meant that many of the insects had not yet reached pupation by day 5 and mortality did not stabilise until day 12. In contrast, *Bt* ssp. *aizawai* has an anorectic reaction on susceptible larvae which causes cessation of feeding, generally within 1–3 h of exposure, and death generally results after 1–5 days.[17–19]

When the relationship between mortality induced by the host plant itself (i.e. control mortality) and that caused by the combined effect of the plant and insecticide (at a single dose) (Fig. 2) is compared over the range of plant groups tested, no discernable trend for a proportional increase in combined effects with corresponding increases in control mortality is found. However, the plot of these variables (Fig. 2) which again shows the scattered nature of the *Bt* data, suggests that there is a 'U-shaped' trend in the teflubenzuron data. The implication is that plants exerting intermediate control mortality (approximately in the range 15 to 35%) decrease the toxicity of teflubenzuron compared with highly susceptible plants. Also, plants which are highly susceptible contribute to more vigorous feeding by larvae (see mean leaf damage rating and percentage mortality [controls] for field strain; Table 3) which increases the ingestion rate of surface residues of the insecticide. Consequently, mortality on highly susceptible plant groups would appear to be insecticide-induced. Greater larval 'fitness' (e.g. size) on the susceptible (high status) leaf discs[16,20] must, to some extent, lower the potential toxicity of the toxicant. Such an effect appears to be shown by the reduced toxicity of teflubenzuron to the field strain on the relatively susceptible, younger cv. Wheelers Imperial compared with the more resistant and older cv. Red Drumhead (Table 3).

These complex interactions can be considered conceptually within a model (Fig. 4)[21] where the toxicity of a foliar-applied insecticide to a herbivore, as determined

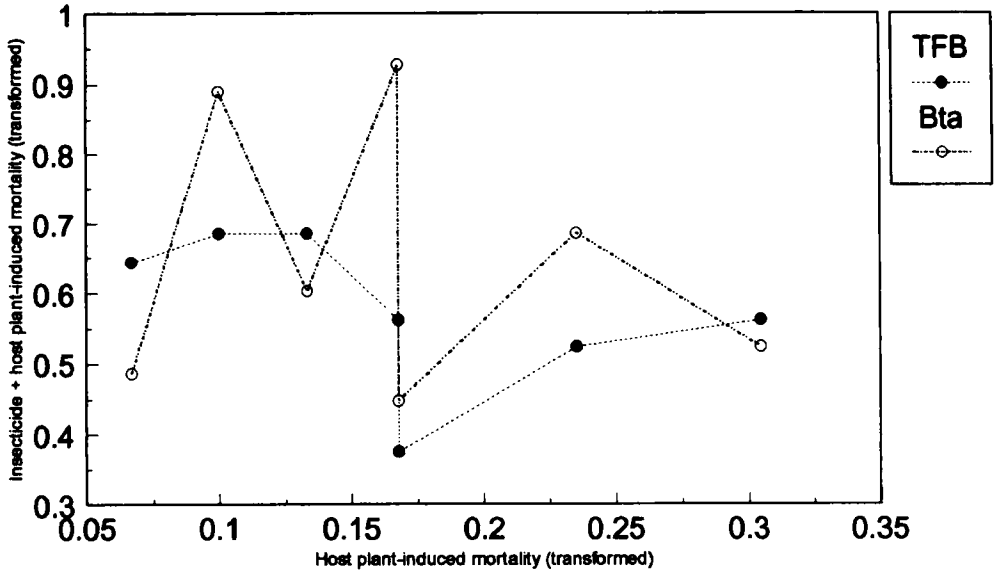


Fig. 2. Non-linear relationship between plant-induced and insecticide[a] plant-induced mortality[b] determined on six plant groups[c] of variable resistance to a laboratory strain (ROTH) of *Plutella xylostella*
[a] TFB = teflubenzuron: Bta = *Bt* ssp. *aizawai*.
[b] End-point mortality data (day 12) arcsine transformed.
[c] As for Fig. 1 and Table 1.

by mortality, is largely a function of the interaction of herbivore fitness, toxicant consumption (in turn dependent on factors such as leaf surface consumption, dilution and possibly biochemical neutralisation) and plant resistance factors (e.g. plant secondary chemicals, poor nutritional quality etc.).

Interactions between high levels of plant resistance and insecticides need not always give rise to higher

TABLE 2
Logit Analysis[a] of Mortality Data for a Laboratory Strain (ROTH) of *Plutella xylostella* with Teflubenzuron and *Bt* ssp. *aizawai* (*Bta*) in Leaf-Dip Bioassays on Three Glasshouse-Grown Host Plant/Cultivar Groups of the Same Age[b]

Insecticide	*Host plant/ cultivar*[c]	*Control mortality*[d] (%) *(day 12)*	LC_{50} [e,f]	*95% FL*	*Slope*[f] (±*SE*)	*RT*[g]
Teflubenzuron						
	TT	10·0a	0·06ab	0·03–0·20	1·63 (±0·56)a	2·7
	WI	13·3a	0·06a	0·04–0·09	3·75 (±0·71)b	2·7
	RD	23·3a	0·16b	0·10–0·40	2·80 (±0·65)b	1
Bta						
	TT	as above[h]	0·02a	0·02–0·03	3·92 (±0·78)a	5·0
	WI		0·02a	0·01–0·03	4·33 (±0·87)a	5·0
	RD		0·10b	0·04–0·7	1·14 (±0·54)b	1

[a] Control mortalities based on those for TT[3]: end-point day 12.
[b] Plant age: 69 days from sowing.
[c] TT = *B. pekinensis* cv. Tip Top: WI and RD = *B. oleracea* var. *capitata* cvs Wheelers Imperial and Red Drumhead respectively.
[d] Control mortality for cv. TT used as correction factor.[15] Significance test: χ^2 with binomial errors ($\chi^2 = 2{\cdot}1$, 2 df, $p > 0{\cdot}05$).
[e] Units: μg AI ml^{-1} for teflubenzuron: iu mg^{-1} for *Bt* spp. *aizawai*.
[f] For each insecticide. LC_{50} values followed by a common letter are not significantly ($P > 0{\cdot}05$) different (five insects/replicate; six replicates/treatment: $n = 30$).
[g] Relative toxicity factor for each insecticide: toxicity (measured by LC_{50}) of specific plant/insecticide combination in relation to least toxic plant/insecticide combination (=cv. Red Drumhead for both TFB and *Bta*).
[h] Controls shared between treatments.

TABLE 3

Logit Analysis of Mortality Data (day 12) and Leaf Damage Consumption Rates (day 5) for a Field Strain (SERD 2) of *Plutella xylostella* with Teflubenzuron and *Bt* ssp. *aizawai* (*Bta*) in Leaf-Dip Bioassays on Two ages of two Glasshouse-Grown *Brassica oleracea* var. *capitata* Cultivars

			Control data					
Insecticide	*Cultivar*[a]	*Plant age (days)*	*Mortality (%) (day 12)*	*Mean leaf damage rating*[b,d] *(day 5)*	LC_{50}[c,d]	*95% FL*	*Slope*[d] *(±SE)*	*RT*[e]
Teflubenzuron								
	WI	84	13·3a	3·8 (±0·2)a	0·10ab	0·01–0·35	0·74 (±0·22)a	4·4
		147	20·0ab	3·7 (±0·2)a	0·44bc	0·08–1·48	0·77 (±0·22)a	1
	RD	84	20·0ab	3·7 (±0·2)a	0·20bc	0·04–0·53	0·97 (±0·23)a	2·2
		147	36·7b	2·5 (±0·2)b	0·05a	0·01–0·19	0·78 (±0·22)a	8·8
Bta								
	WI	84	as above[f]		0·11a	0·04–0·21	1·38 (±0·39)a	5·2
		147			0·57b	0·28–3·03	1·28 (±0·39)a	1
	RD[g]	84			>1	—	—	<1
		147			0·11a	0·06–0·18	0·06 (±0·18)b	5·2

[a] WI and RD = *B. oleracea* var. *capitata* cvs Wheelers Imperial and Red Drumhead respectively.
[b] Based on rating scale (Section 2.6).
[c] Units: μg AI ml^{-1} for teflubenzuron: iu mg^{-1} for *Bt* ssp. *aizawai*. Not corrected for control mortality.
[d] For each insecticide (or controls), values followed by a common letter are not significantly ($P > 0·05$) different (five insects/replicate: six replicates/treatment: $n = 30$).
[e] Relative toxicity factor for each insecticide: toxicity (measured by LC_{50}) of specific plant/insecticide combination in relation to least toxic plant/insecticide combination.
[f] Controls shared between treatments.
[g] Inadequate response at concentrations tested prevented logit analysis.

levels of mortality than interactions with intermediate resistance or susceptible plants. Such incompatibility of plant resistance and insecticides has been shown in a variety of systems. For example, *Helicoverpa zea* (Boddie) has been shown to have tolerance for the insecticide carbaryl following ingestion of 2-tridecanone from tomatoes (glandular trichomes), owing to induction of its monooxygenase detoxification system.[22] Similarly, gossypol from resistant cotton, enhances the production of *N*-demethylase which can also detoxify

TABLE 4

Selected Regression Data for Sum of Leaf Damage Rating[a] (Response Variable) against Log Dose (+1) for Teflubenzuron and *Bt* ssp. *aizawai* (*Bta*) using binominal errors[b] (Fig. 3A & B)

Insecticide	*Cultivar*[c]	*Plant age (days)*	r^2	*Intercept (a) ±SE*	*Slope (b) ±SE*	*F*	*P*
Teflubenzuron	WI	84	0·63	1·97 (±0·37)a	−2·43 (±0·71)ab	12·8	<0·05
		147	0·90	1·86 (±0·35)a	−2·74 (±0·70)ab	17·1	<0·05
	RD	84	0·85	3·43 (±0·60)a	−4·07 (±0·93)b	24·0	<0·05
		147	0·73	0·82 (±0·28)a	−2·00 (±0·65)a	10·4	<0·05
Bta	WI	84	0·59	2·11 (±0·37)a	−4·84 (±2·00)a	5·7	ns
		147	0·77	1·51 (±0·30)a	−5·29 (±1·80)a	8·9	ns
	RD	84	<0·1	1·71 (±0·34)a	0·22 (±2·33)a	<1	ns
		147	0·80	0·18 (±0·25)b	−3·72 (±1·78)a	4·7	ns

For each toxicant, values followed by a common letter are not significantly ($P > 0·05$) different (five insects/replicate: six replicates/treatment: $n = 30$).
[a] Field strain (SERD 2) of *Plutella xylostella*.
[b] Analysed by multiple regression in GLIM using a binomial best-fit model: sum of leaf damage rating (as a binomial) = $a + b$ (log dose + 1).
[c] WI = *B. oleracea* var. *capitata* cv. Wheelers Imperial: RD = *B. oleracea* var. *capitata* cv. Red Drumhead.

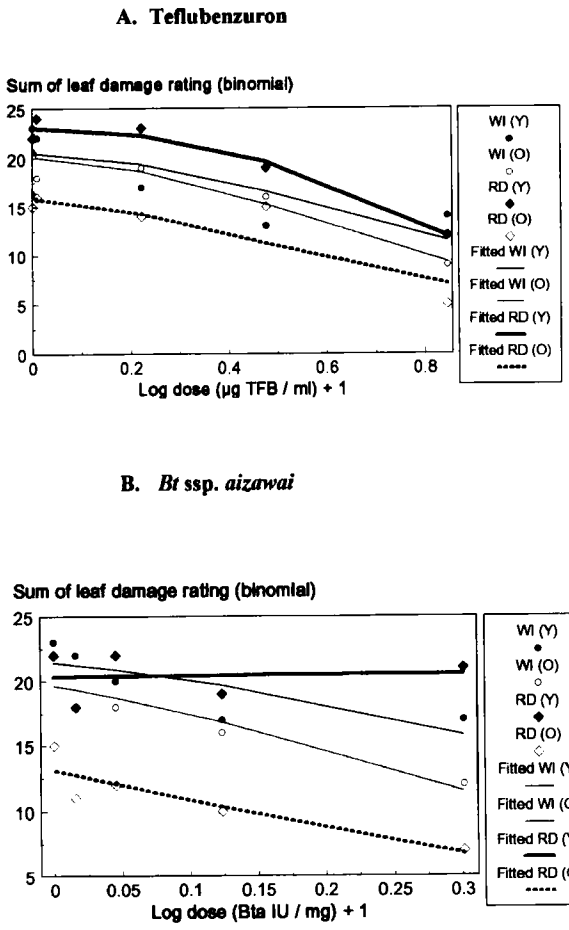


Fig. 3. Relationship[a] between larval damage by a field strain (SERD 2) of *Plutella xylostella* to the surface of leaf discs from four plant groups[b] and the concentration of teflubenzuron or *Bt* ssp. *aizawai*

[a] See Table 4 for selected regression data.

[b] WI (Y) = cv. Wheelers Imperial, 84 days old: WI (O) = cv. Wheelers Imperial, 147 days old: RD (Y) = cv. Red Drumhead, 84 days old: RD (O) = cv. Red Drumhead, 147 days old.

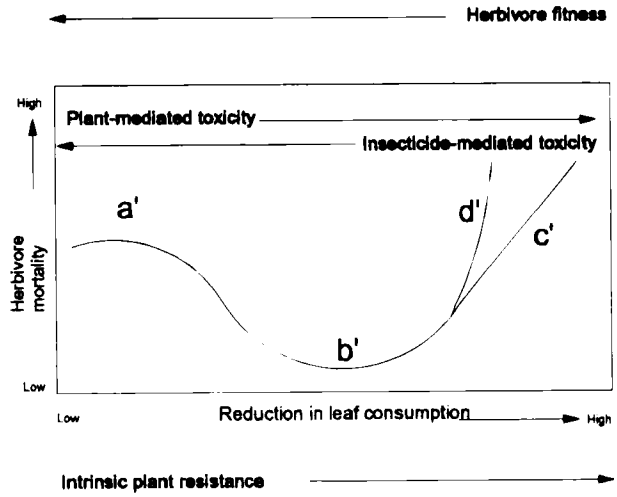


Fig. 4. Conceptual model showing some possible ditrophic (plant–macrophagous herbivore) interactions with a foliar-applied, stomach-acting insecticide (from Wright & Verkerk, Ref. 21).

[a'] High level of herbivore fitness on highly susceptible plants reduces potential toxicity of insecticide (tolerance): consumption rate of treated leaf discs directly controls dose of toxicant ingested. Contact toxicity (where appropriate) may also be greater on such susceptible leaves.

[b'] Point of minimum impact on herbivore of combined effects of plant resistance factors and insecticide.

[c'] Additive effect of plant resistance factors and insecticide.

[d'] Synergistic interaction of plant resistance factors and insecticide.

xenobiotic agents.[23] In the present study, the approximately nine-fold greater susceptibility of the field strain of *P. xylostella* to teflubenzuron when supported on mature cv. Red Drumhead compared with mature cv. Wheelers Imperial would argue against any elevation of microsomal monoxygenases in the more insecticide-tolerant field strain (Table 3). In this case, since there is little firm evidence for a powerful antibiosis mechanism in *B. oleracea* var. *capitata*,[20] the effects of differential feeding rates and dilution may be more important (see below).

Logit analysis of mortality data, in contrast to comparisons of single (median) doses, allowed effects of several concentrations of the insecticides to be assessed on various plant groups: the most robust feature of logit analyses is the resulting LC_{50} values, whereas there is some controversy over the biological significance of the slopes.[24] Logit analysis of data from all experiments (Tables 1, 2 and 3) supported the trends both found by a comparison of single doses (Figs 1 and 2) and explained in the conceptual model (Fig. 4). The essential trend noted in these analyses is that toxicity (LC_{50}) of insecticides to *P. xylostella* tends to be lowest with plants exhibiting some degree of resistance and increases as the intrinsic plant resistance either increases or decreases thereby showing a 'U-shaped' trend. Such a trend was not evident for the experiment using young (69-day-old) plants, as none of the plants offered substantial resistance (Table 2). As a result they reflected only the left side of the 'U'. Similarly, the right side of the 'U' was not as well represented as it might have been due to the lack of highly resistant plant groups in the experiments. Interestingly, there were some similarities in trends between teflubenzuron and *Bt* ssp. *aizawai* (Tables 2 and 3), implying that there are likely to be some processes in common in their interactions with the herbivore–host plant system. For example, although not measured in the present study, insects reared on more resistant plant groups are generally smaller[1,4,6,25] and are thus more susceptible to the effects of given doses of insecticides.

A further interaction, only considered indirectly in the present study, concerns the relationship between relative amounts of toxicant and food ingested by the herbivore. Larvae on the most resistant plant groups tested (e.g. mature cv. Red Drumhead) tended to consume considerably more (*c.*5-fold) leaf biomass than

larvae on susceptible plant groups,[20] a finding consistent with studies by other workers,[26–31] while the relationship between plant resistance and the leaf surface area consumed tended to be exactly converse to that of plant resistance and biomass consumption. Thus, larvae on more resistant leaf discs consumed considerably less of the surface area but fed much more deeply within the leaf than did larvae on susceptible leaf discs, this effect being noted both in the presence and the absence of insecticides (Tables 2 and 3). When a toxicant, of a type which does not impose either an anorectic or antifeedant effect and has no appreciable translaminar activity, is applied to the surface of a leaf (or leaf disc), the implications are clear: the ratio of insecticide to leaf biomass consumed (assuming equivalent bioavailability) will tend to be much greater on a susceptible than on a resistant host plant.

In the above case, the bulk of leaf tissue consumed by larvae may serve to dilute the insecticide. However, working against the benefits of dilution on more resistant plants are the factors which contribute to low herbivore fitness and, presumably, increase susceptibility to xenobiotics such as insecticides. In contrast, larvae on insecticide-treated susceptible plants, although ingesting greater amounts of foliar insecticide with relatively little host-plant bulk to aid dilution, have the benefit of high food quality and consequent fitness to assist in combating the toxic effects of the insecticide. It is perhaps some of these complex and often conflicting interactions which contribute to the wide array of responses noted in previous studies of plant resistance–insecticide interactions.[2–11]

The above relationships appeared to be less clearcut with the *Bt* product tested. The fundamental difference in the mode of action of *Bt* compared with teflubenzuron probably accounts for the lack of significance of regression model fits for all the four plant groups tested with *Bt* ssp. *aizawai* when leaf damage rating was regressed against insecticide concentration. The results implied that the *Bt* concentration had no significant bearing on leaf surface area damage (i.e. slopes of the best-fit lines were not significantly different from zero) although leaf discs treated with *Bt* ssp. *aizawai* from the most susceptible plant groups still tended to be more toxic than those from 'intermediate' plant groups. Such an effect may be related to the higher rate of ingestion of *Bt* by larvae on highly susceptible and preferred plant material. Differences in secondary plant substances between the various plant groups may also have had contrasting effects on the action of the *Bt* toxin.

The substantially lower leaf-damage ratings with *Bt* on more resistant plants is shown by the significantly lower intercept (*a*) of the regression between leaf damage and *Bt* concentration of mature cv. Red Drumhead, compared with the other three plant groups (Fig. 3 and Table 4). The greater toxicity of all doses of *Bt* on cv. Tip Top treated with the higher concentration (100 μl AI ml^{-1}) of the surfactant 'Triton'-X 100 may have been associated with differences in the distribution of *Bt* residues with the two surfactant concentrations, *Brassica pekinensis* leaves dipped in this higher concentration being covered in a more continuous and even film of insecticide. Although this might imply that residue avoidance by larvae contributed to the increased toxicity of *Bt* with the higher surfactant concentration, studies by Hoy & Hall[32] on the behaviour of *P. xylostella* when confronted with *Bt* or esfenvalerate droplets, showed little evidence of avoidance. Increased response at the higher surfactant concentration was more likely to be associated with the greater probability of larvae ingesting a lethal dose of *Bt* prior to the onset of an anorectic reaction. With teflubenzuron, where significant effects were not as apparent, the majority of the leaf tissue was consumed (over five days) and differences in residue deposition were perhaps less important.

The present studies have highlighted some of the complexity of interactions between plant resistance, insecticides and macrophagous larvae. Sublethal effects on insects[33–35] (other than feeding), possible differences in susceptibility between sexes and effects of very high levels of plant resistance remain to be examined.[36]

Extended end-points (i.e. 12 days cf. the three to five days more often used) were selected in the present experiments in order to detect more fully the impact of the various host-plant/pesticide treatments on mortality of *P. xylostella*. These laboratory studies have suggested that insecticides applied to *B. oleracea* var. *capitata* offering partial plant resistance (>30–40% mortality in the absence of insecticides) may contribute to improved control of *P. xylostella* compared with highly susceptible plants. Such control, mediated by the combined (additive or synergistic) effects of plant resistance factors and insecticide toxicity, is desirable and may allow substantial reductions in insecticide input.[1,24] The present studies also suggest that plant groups offering slightly reduced susceptibility compared with the most susceptible plant groups may actually decrease the efficiency of insecticides relative to the susceptible plant groups. This effect, evident with both teflubenzuron and *Bt* ssp. *aizawai*, was probably a result of reduced insecticide/leaf disc consumption rates by larvae on the less susceptible plant groups, which in turn did not contribute to any appreciable plant-mediated loss of fitness. Such interactions warrant field-testing.

In the field, there is a much greater array of possible variables, and interactions will be more complex than in the laboratory. Ultimately, it is the net damage to the plant which is most important from a pest management viewpoint. For this reason, field experiments emphasising some of the mechanisms suggested in the present study, such as toxicant ingestion rates, herbivore fitness, plant resistance mechanisms and insecticide mode of action, would be invaluable. Collection of such data

should assist in the development of appropriate and realistic models which would contribute to our understanding of the complex and interacting processes involved and, in turn, improve compatibility between host plants and selective insecticides in IPM programmes.

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